

(GC) represents a particular vulnerability and predictor of relapse in ETP and some non-ETP T-ALL and that removal of IL-7 or JAK inhibition resensitized GC-resistant, IL-7-dependent T-ALL to GC.<sup>8</sup> Mechanistically, GC exposure directly causes upregulation of IL-7R expression, leading to downstream BCL2 upregulation, which in turn promotes leukemia survival. However, targeted inhibition of the IL-7R/JAK/STAT5/BCL2 signaling axis could reverse this phenomenon, recover GC sensitivity, and synergistically provide therapeutic benefit in T-ALL.<sup>9</sup> These data suggest that dual inhibition combined with GC-rich treatment phases could potentially abrogate GC resistance in select cohorts. However, as in any aggressive and heterogeneous cancer, single-cell data have already introduced the concern that select T-ALL cell populations with variable responsiveness to inhibition may persist at relapse.<sup>10</sup> Thus, the field will certainly benefit from efforts to combine synergistic IL-7R pathway-targeted therapies in a multifaceted attempt to avoid therapeutic escape, as here introduced by Courtois et al.

**Conflict-of-interest disclosure:** The authors declare no competing financial interests. ■

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## LYMPHOID NEOPLASIA

Comment on *Li et al*, page 172

# All about Down syndrome ALL

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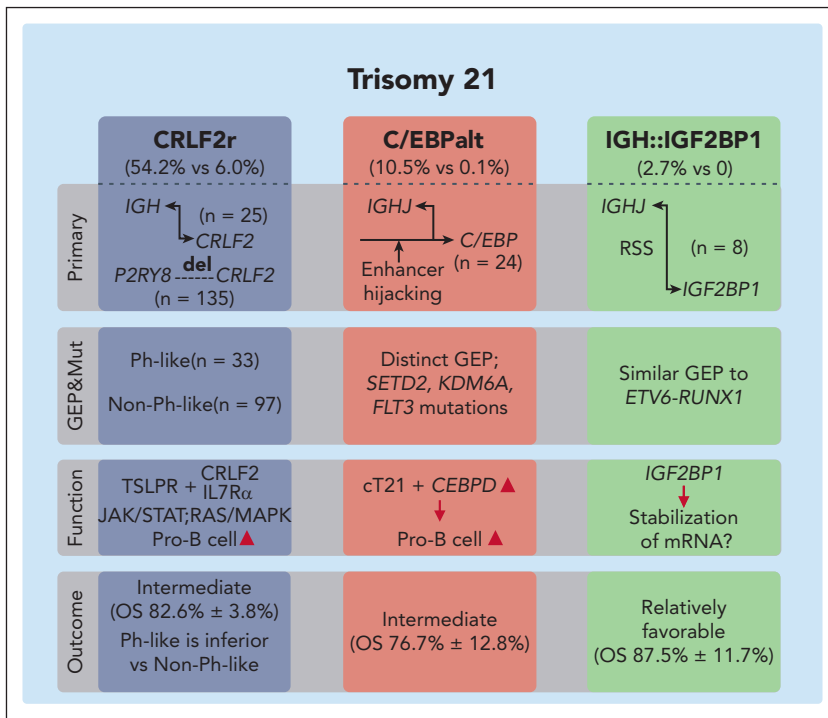
**Children with Down syndrome (DS) have a 20 times higher risk of developing acute lymphoblastic leukemia (DS-ALL). In this issue of *Blood*, Li et al<sup>1</sup> report a comprehensive genomic analysis of 295 DS-ALLs, identifying 15 distinct molecular subtypes, 3 of which (see figure) have a much higher frequency in DS-ALL compared with ALL in children without DS (non-DS ALL).**

Chromosomal aneuploidy is common in cancer. Although DS is surprisingly associated with an overall decreased lifetime risk of cancer, the risk for childhood leukemias is markedly increased.<sup>2</sup> Myeloid leukemias of DS have unique somatic genomic features; however, DS-ALLs are more heterogeneous, but with significant differences from non-DS ALL in children.<sup>3</sup> We and others have discovered that approximately half of DS-ALLs have an abnormal expression of CRLF2, whose heterodimerization with IL7Rα forms the receptor to thymic stromal lymphopoietin. CRLF2 expression is caused by chromosomal rearrangement juxtaposing the upstream promoter of a constitutively expressed gene *P2RY8* or by a translocation of CRLF2 into the immunoglobulin heavy chain locus (IGH). CRLF2 rearrangements (CRLF2r) are frequently accompanied by additional activating mutations in the receptors themselves or in the downstream signaling molecules of JAK/STAT or RAS/MAPK pathway (reviewed in Tal et al<sup>4</sup>). Interestingly, the same type of ALL has been detected in ~5% of non-DS ALL.<sup>5</sup>

In addition to previously described CRLF2r DS-ALL, Li et al discovered 2 other

subtypes of ALL enriched in DS-ALLs, altered C/EBP (C/EBPalt) and *IGH::IGF2BP1*. More than 10% of DS-ALL cases harbor alterations targeting C/EBP genes, with overexpression of *CEBPD*, or less commonly, *CEBPA*, or *CEBPE*. The C/EBP family of transcription factors regulate genes involved in multiple biological processes. They also play a crucial role in myeloid differentiation and pathogenesis of myeloid and lymphoid malignancies.<sup>6</sup> The authors reported a significant concurrence of *FLT3*, *SETD2*, and *KDM6A* mutations (42.3%, 42.3%, and 30.8%, respectively) in C/EBPalt DS-ALL, compared with only 4.1%, 5.0%, and 5%, respectively, in the rest of DS-ALL subtypes. *CEBPD*, the most commonly altered gene in C/EBPalt subtype, enhanced the differentiation of mouse hematopoietic progenitor cells into pro-B cells in vitro, particularly in a DS genetic background.<sup>1</sup> This finding is consistent with a specific role of *CEBPD* overexpression in the development of DS-ALL.

Another novel subtype defined by *IGH::IGF2BP1* rearrangements was observed in 2.7% of DS-ALL cases. This subtype is characterized by deregulated expression of *IGF2BP1* gene, which



Molecular complexity of DS-ALL. Li et al identified 15 molecular subtypes, with 3 significantly novel subtypes enriched in DS-ALL compared with non-DS ALL (ratios shown in parentheses under subtype labels). CRLF2r, commonly resulting from IGH::CRLF2 translocations or P2RY8::CRLF2 microdeletions, leads to CRLF2 overexpression and activation of the JAK/STAT or RAS/MAPK signaling pathway, promoting pro-B-cell proliferation. Although CRLF2r is a signature event of a Ph-like subtype, this large DS-ALL cohort reveals further heterogeneity based on GEP, with Ph-like CRLF2r cases having worse clinical outcomes than non-Ph-like group. Another novel subtype is characterized by C/EBP gene family activation, primarily involving the CEBPD gene through genomic translocations (often with the IGHJ region) or enhancer hijacking mutations. This subtype displays a unique GEP, higher mutation rates in SETD2, KDM6A, and FLT3 genes, and intermediate risk levels. CEBPD overexpression promotes hematopoietic progenitor cell differentiation into pro-B cells, particularly in a cT21 genetic background. A minor subtype, characterized by IGHJ::IGF2BP1 gene rearrangement likely through RAG-mediated structural changes near RSS regions, has a relatively favorable clinical outcome. Although no distinct GEP is observed for this subtype, some cases share a similar GEP with the ETV6::RUNX1 subtype, potentially because of IGF2BP1 overexpression in ETV6::RUNX1 subtype resulting from ETV6 loss, which normally represses IGF2BP1 expression. The observed function of IGF2BP1 in stabilizing ETV6::RUNX1 messenger RNA further supports the potential association between these 2 genetic alterations in ALL. However, as ETV6::RUNX1 messenger RNA is not expressed in DS-ALL, the role of IGF2BP1 in DS-ALL remains unknown. ALL, acute lymphoblastic leukemia; CRLF2r, CRLF2 rearrangement; GEP, gene expression profile; RAG, recombination-activating gene; RSS, recombination signal sequences.

encodes a member of the insulin-like growth factor 2 messenger RNA (mRNA)-binding protein family. This protein is required for the transportation of certain mRNAs by affecting their stability, translatability, or localization. In ETV6::RUNX1 ALL, it binds and stabilizes ETV6::RUNX1 mRNA.<sup>7</sup> IGF2BP1 is also activated in ETV6::RUNX1-like ALL, owing to the loss of ETV6, a transcription repressor of IGF2BP1. Further research is needed to evaluate IGF2BP1 binding partners in non-ETV6::RUNX1 ALL and understand why this subtype is more frequent in DS-ALL.

The current and earlier studies raise 2 interesting questions. The first relates to the poor clinical outcome of DS-ALL. The worse prognosis of DS-ALLs has been attributed to both the high risk of

chemotherapy associated infections in these patients and to the genomic subtypes of DS-ALLs. Here, Li et al demonstrate that this higher risk is limited to a subgroup of CRLF2r ALL that has a gene expression signature of Philadelphia chromosome (Ph)-like subtype of ALL. However, what distinguishes the Ph-like subtype that might explain the worse prognosis is still unknown.

Somatic alterations in CRLF2r ALL differed markedly between Ph-like and non-Ph-like subtypes with IKZF1 (76.9% vs 16.7%), XBP1 (26.9% vs 0%), USP9X (34.6% vs 2.8%), and EBF1 (53.8% vs 2.8%) alterations overrepresented in the former. However, by contrast to a previous study,<sup>8</sup> IKZF1 deletions were not independently associated with worse prognosis. Interestingly,

we have shown that CRLF2r JAK2 mutated DS-ALLs are quite sensitive to chemotherapy, possibly because of “hyper-signaling” by mutated JAK2 in the B-cell blasts.<sup>3</sup> The USP9X mutant, which is more common in Ph-like subtype, reduces this hypersignaling and enhances the resistance of leukemic blasts to chemotherapy.

Perhaps the greatest mystery is why constitutive trisomy 21 (cT21) confers a significantly higher risk of B-cell precursor ALL. Recent analysis of hematopoietic development in human fetuses with DS revealed a marked B-cell developmental arrest.<sup>9</sup> Li et al demonstrate that aberrant CEBPD expression enhances pro-B-cell development in the background of cT21. We have recently reported the same phenomenon with CRLF2 + IL7R expression in human cells.<sup>10</sup> The relative block in B-cell differentiation may also explain the high rate of recombination-activating gene mediated genomic rearrangements identified by Li et al, leading to each of the genomic abnormalities (see figure). It is tempting to speculate that DS-ALL represents an unintended consequence of genomic events that drive differentiation toward the B-cell lineage, thereby rescuing the inherent developmental defect in DS. This phenomenon is somewhat similar to the myelodysplastic syndrome arising from the bone marrow “attempt” to correct the germ line SAMD9/SAMD9L mutation by deleting the chromosome 7 carrying the mutated gene.<sup>11</sup> Could DS-ALL be a disease caused by an attempt to correct another disease, such as the B-cell developmental arrest caused by 3 copies of chromosome 21?

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## RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Yu et al, page 185

# Ironing out $\beta$ -thalassemia during pregnancy

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**In this issue of *Blood*, Yu et al<sup>1</sup> elucidate the mechanisms of iron homeostasis in  $\beta$ -thalassemia during pregnancy. The inherited disorder  $\beta$ -thalassemia is caused by disruptions to hemoglobin  $\beta$ -globin chain production, resulting in microcytic, hypochromic anemia. Individuals with  $\beta$ -thalassemia major have little to no production of  $\beta$ -globin chains, which leads to a more severe transfusion-dependent anemia than individuals with  $\beta$ -thalassemia intermedia. The severity of  $\beta$ -thalassemia intermedia is highly variable, with the severity of the specific globin mutation and the proportion of  $\beta$ -globin chain produced determining the severity of the condition.<sup>2</sup> Iron overload is a common feature in both  $\beta$ -thalassemia major and intermedia due to increased erythropoietic demand and suppression of hepcidin expression.**

Although transfusion contributes significantly to iron load in  $\beta$ -thalassemia major, enhanced gastrointestinal absorption is central to tissue iron accumulation  $\beta$ -thalassemia intermedia.<sup>3</sup> Given this background, it is important to consider the potential impact of gestation on  $\beta$ -thalassemia. Patients with both transfusion- and non-transfusion-dependent thalassemia may experience complications during pregnancy, including cardiac and liver issues, increased risk of thrombosis and infection, endocrinopathies, and medication adverse effects.<sup>4,5</sup>

Using murine models of  $\beta$ -thalassemia intermedia (Th3/+), the article investigates changes in iron metabolism in both dams and fetal offspring during gestation. This

study is unique as it provides an investigation into the pathophysiology of iron balance, which has not yet been reported in the literature. The authors discovered that hyperferremia in pregnant  $\beta$ -thalassemia mouse models resulted in differential iron loading of fetuses compared with pregnant wild-type counterparts. To understand the iron loading differential, expression of iron transporters and proposed possible mechanisms that could account for this disparity were investigated. Yu et al also examined the impact of fetuses' genotype, thalassemia or wild type, on thalassemic dams, and their findings suggest a fetal influence on maternal iron homeostasis. Their experimental design included both wild-type and  $\beta$ -thalassemia (Th3/+) dams carrying

fetuses of both genotypes, providing a unique perspective because the current literature primarily focuses on  $\beta$ -thalassemic mothers.

Yu et al discovered that both nonpregnant and pregnant Th3/+ mice have increased splenic iron load compared with wild-type controls, which aligns with the known functions of the spleen as a site of extramedullary erythropoiesis and red blood cell recycling. They also showed that pregnant wild-type and Th3/+ mice have enhanced iron absorption compared with nonpregnant mice of the same genotype. However, they found similar iron absorption in nonpregnant wild-type and  $\beta$ -thalassemia intermedia mice. The authors discuss that iron absorption normalizes in weanlings and adolescent Th3/+ mice, which may present a discrepancy between humans as iron loading increases with age.<sup>6</sup>

Yu et al found that Th3/+ fetuses from both wild-type and Th3/+ dams had iron overload, suggesting that iron loading occurs early in pregnancy. Late gestational iron absorption studies demonstrated lower absorption in both wild-type and Th3/+ fetuses of Th3/+ dams compared with those of wild type, suggesting that gestational iron loading mainly occurs early in pregnancy. The results presented in the article delineate a crucial gestational window for iron loading. However, the use of chelation medications during pregnancy is generally considered unsafe due to teratogenicity. Case studies and retrospective analysis have shown successful pregnancies in patients with unintentional use; however, due to lack of controlled studies in humans, discontinuation of these medications at the onset of pregnancy is recommended.<sup>4,7</sup>

The authors also found significant changes in the expression of nonheme iron transport proteins (divalent metal transporter 1 [DMT1], transferrin receptor 1 [TFR1], and ferroportin 1 [FPN1]) in various organs of both pregnant and nonpregnant Th3/+ dams, compared with wild-type control mice. Their study revealed that the iron importer DMT1 and exporter FPN1 expression in the duodenum was lower in pregnant Th3+ dams than in nonpregnant Th3/+ dams. Furthermore, their data suggest that iron-loaded fetuses were able to mitigate iron loading through hepcidin transactivation and downregulation of